Experience with the Test for Vi Agglutinative Properties for *Eberthella typhosa*

MARION B. COLEMAN, F.A.P.H.A.

Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

THE study of Vi agglutination [1] with *Eberthella typhosa* was undertaken with the twofold objective of securing experience with the technical procedures and determining the practicability of performing the test as a routine procedure in a diagnostic laboratory. Vi agglutination has been found of limited value in the diagnosis of typhoid fever, since it does not occur consistently at any one stage of the illness.[2-9] On the other hand, it has been demonstrated in sera from a high percentage of typhoid carriers.[10-14] Most of these reports are based on reactions with a pure Vi strain of *E. typhosa,* designated by Bhatnagar [12] as "Vi 1," or with a strain rich in Vi antigen after absorption of the serum with a strain devoid of Vi antigen. No mention was found in the literature of a zone of inhibition (prezone, prozone, or proagglutinoid zone) in the tests, a phenomenon that was at once apparent when our work was undertaken. Centrifugation proved effective in demonstrating reactions in high concentrations of sera, not only of those shown to have zones of inhibition, but in many in which no agglutination was observed prior to this treatment. Later, it was found that Shibley (1929)[15] had made a similar observation in his study of artificially produced zones of inhibition.

TECHNIC

A pure Vi strain of *E. typhosa,* designated by Bhatnagar as "Vi 1," and the "Watson" strain, known to be rich in Vi antigen, were obtained from Dr. Kauffmann, State Serum Institute, Copenhagen, Denmark. They were maintained on inspissated egg medium,[16, 17] in sealed tubes and stored in a refrigerator.

INSPISSATED EGG MEDIUM [17]

Sodium chloride solution, 0.5 per cent.. 1 part
Eggs (white and yolk)..... 3 parts

Transfer the egg contents aseptically to a weighed sterile flask containing glass beads. Determine the increase in weight and add the required amount of sterile salt solution. Mix by shaking thoroughly. Dispense aseptically 3 ml. in 125 x 13 mm. tubes. Slant and inspissate at 80° C. for 1 hour on two successive days.

Transplants were made at 6 month intervals. Subcultures on beef infusion agar were incubated from 18 to 20 hours at from 35° to 37° C. and suspended in 0.85 per cent salt solution. Living suspension was prepared each day that tests were performed. Suspension killed by the addition of 0.2 per cent formalin (40 per cent formaldehyde) was stored in a refrigerator and used for no longer than 4 weeks after preparation. The turbidity of suspensions was adjusted to that of barium sulfate standard No. 3.[18] Equal volumes (0.3 ml.) of suspen-

sion and 5-, 10-, 20-, and 40-fold dilutions of sera were combined in 11 x 75 mm. tubes, incubated for 2 hours at from 35° to 37° C., and left in a refrigerator overnight. The reactions were recorded before and after centrifugation for 10 minutes at approximately 2,000 r.p.m.

The degree of agglutination was recorded as follows:

A. Before centrifugation—
- 4+ supernatant clear; complete agglutination
- 3+ supernatant clear or nearly clear; definite clumping
- 2+ supernatant slightly turbid; definite clumping
- \+ supernatant turbid; small clumps definitely visible to unaided eye
- — uniformly turbid suspension; no clumps

B. After centrifugation—all reactions read after shaking.
Comparison with the suspension combined with salt solution for purposes of control is very important.
- 4+ complete agglutination in clear fluid
- 3+ definite agglutination in clear or nearly clear fluid
- 2+ definite agglutination in slightly turbid fluid
- \+ clumps definitely visible to the unaided eye in turbid fluid
- — no clumps; uniformly turbid suspension

Serum for purposes of control was produced in rabbits with *E. typhosa* Vi 1. The titer was relatively low. After absorption with *E. typhosa* "Felix H 901," a strain devoid of Vi antigen, no agglutination was obtained with the latter in a 10-fold or greater dilution, while the Vi 1 strain was agglutinated in an 80- or 160-fold dilution. A high titered agglutinating serum that contained no Vi agglutinative properties was also used for purposes of control.

Absorption of agglutinative properties other than Vi was undertaken with a small number of sera. A heavy suspension of the growth of *E. typhosa* Felix H 901 from beef infusion agar was made in a 5-fold dilution of serum. This was incubated 2 hours at from 35° to 37°C. and left at room temperature overnight. It was then centrifuged until the supernatant was clear—usually for 30 minutes at approximately 2,000 r.p.m. The supernatant, undiluted and in 2-, 4-, and 8-fold dilutions, was tested for agglutination with *E. typhosa* Felix H 901 and Vi 1, and usually with the Watson strain.

DISCUSSION

The results summarized in Table 1 were obtained after centrifugation of tests with living suspension of *E. typhosa* Vi 1. All sera were tested at least twice, except a few that reacted in high dilutions in the first examination. The relatively large number of

TABLE 1

Reactions in Sera Following Centrifugation of Tests with Living Suspension of E. typhosa Vi 1

Source of Specimen	Number Tested	Definite Agglutination		No Agglutination		Irregular or Indefinite Agglutination	
		No.	Per cent	No.	Per cent	No.	Per cent
Typhoid carriers	82	62	75.6	8	9.7	12	14.6
Patients having typhoid fever	37	14	37.8	12	32.4	11	29.7
Patients having infections with members of the paratyphoid-enteritidis (Salmonella) group	12	1	8.3	8	66.6	3	25.0
Individuals who had received typhoid vaccine	46	7	15.2	34	73.9	5	10.8
Random specimens*	157	8	5.1	130	82.8	19	12.1

* From the information available, these specimens could not be placed in any of the other four groups.

indefinite or irregular reactions should not be misinterpreted. They usually represent no agglutination in one test and definite but incomplete agglutination (2+) in a 10-fold dilution of the serum in another test, which is no greater variation than may be expected in repeated examinations of this type. Agglutination was apparent only after centrifugation of the tests with half of the 92 sera that gave reactions.

them, as well as in the artificially produced Vi agglutinating serum. Thus, it seems important that tests for Vi agglutination be performed at least twice, preferably with not less than 3 or 4 days intervening. The results with two sera, one from a patient having typhoid fever and the other from a carrier, have been summarized in Table 2 to illustrate this point.

Since some species of Salmonella

TABLE 2

Vi Agglutination in Two Sera Tested at Intervals

			Agglutination											
			Before Centrifugation						After Centrifugation					
Serum	*Date Received*	*Date Tested*	*1:10*	*1:20*	*1:40*	*1:80*	*1:160*	*1:320*	*1:10*	*1:20*	*1:40*	*1:80*	*1:160*	*1:320*
No. 2213 from typhoid carrier	8- 5-41	8- 6-41	+	+	—	—	..	..	+	+	+	—	..	..
		8- 7-41	2+	3+	+	—	..	..	3+	3+	3+	+	..	..
		8-13-41	2+	2+	—	—	..	..	3+	3+	2+	—	..	..
No. 1370 from a patient having typhoid fever	5-20-41	5-20-41	—	—	—	—	+	+	4+	4+	4+	4+	3+	3+
		5-23-41	4+	3+	3+	3+	+	—	4+	4+	4+	4+	3+	—

4+ = complete agglutination
3+, 2+, and + = degree of agglutination as compared with 4+
— = no agglutination

Approximately one-third of the sera were tested with formalin-treated as well as with living suspension of *E. typhosa* Vi 1. The former was agglutinated somewhat more frequently before centrifugation, but the titer after centrifugation was often higher with living suspension.

Not infrequently, sera that reacted only after centrifugation when first tested, gave agglutination before as well as after this treatment in tests performed one or more days later. Occasionally, little or no agglutination occurred before or after centrifugation of tests with fresh sera, and definite reactions occurred in later tests. These irregularities cannot be explained by variation in the agglutinability of the living culture, since usually several sera were tested at the same time and agglutination was obtained in some of

possess Vi antigen, it is not surprising that serum from one patient having an infection with a member of group D Salmonella gave definite Vi agglutination with *E. typhosa* in an 80-fold dilution. Blood from three other individuals having infections with the same species failed to show any Vi agglutinative properties, while that from a fifth reacted irregularly.

The relatively large number (15 per cent) of reactions in sera from individuals who had received typhoid vaccine may be more apparent than real, because many such specimens that failed to agglutinate were tested only once, and therefore are not included. One of the seven individuals who had received typhoid vaccine and whose sera contained Vi agglutinative properties was a nurse who was caring for her brother, who had typhoid fever.

No *E. typhosa* was found in several fecal specimens from her; no specimens were submitted from the others.

Sufficient information is not available concerning the specimens selected at random to draw definite conclusions as to the significance of the Vi agglutinative properties in eight, or 5 per cent, of the number examined. All except one contained granular or floccular agglutinative properties or both. One fecal specimen only was received from each of two of these individuals, and none from the others. One was said to have had her gall bladder drained, but so far as could be determined, a bacteriologic examination of the bile or of feces had not been undertaken.

The presence of Vi agglutination was not correlated with floccular and granular agglutination. Of the eighty-two sera from carriers, eighty gave floccular agglutination in at least a 40-fold dilution and two, in only a 20-fold dilution. Vi agglutination occurred in one of the latter. Of the other seven in which no Vi agglutinative properties were demonstrated, one gave floccular agglutination in a 640-fold dilution, five in a 160-fold dilution, and one in an 80-fold dilution. Several sera from patients having typhoid fever contained no Vi agglutinative properties, but gave both granular and floccular agglutination with *E. typhosa* in relatively high dilutions.

Forty-five sera absorbed with *E. typhosa* Felix H 901 were tested for agglutination with *E. typhosa* Vi 1, and most of them with the Watson strain as well. Centrifugation was often necessary to demonstrate agglutination after, as well as before, absorption. There was no appreciable difference in titer with *E. typhosa* Vi 1 in unabsorbed and absorbed portions of sera. The titer with *E. typhosa* Watson in absorbed sera was usually the same as, but in a few instances lower than that with Vi 1. This procedure was not studied further, because it seemed to offer no advantage over the simpler agglutination test.

SUMMARY

The observations reported are based on the demonstration of agglutination with the strain of *E. typhosa* designated as Vi 1 by Bhatnagar. Vi agglutination was often demonstrated only after centrifugation, especially in tests with fresh sera. If these sera were retested after a few days, agglutination was sometimes observed before the tests were centrifuged. These facts may be of significance in other agglutination tests, especially in sera with zones of inhibition.

In a study of over 300 sera, definite Vi agglutination was obtained in 75 per cent from known typhoid carriers; 37 per cent from patients having typhoid fever; 15 per cent from individuals to whom typhoid vaccine had been administered; and 5 per cent of sera selected at random. Insufficient data are available to establish the significance of the reactions in the " random " specimens, and in those from persons who had received typhoid vaccine.

The demonstration of Vi agglutination with *E. typhosa* suggests infection with this microörganism and warrants thorough investigation. Absence of Vi agglutination does not exclude such a condition. The performance of this test as a routine diagnostic procedure seems practicable in any laboratory where facilities are available for adequately controlling the reactions of the agglutinating suspension and the sera.

REFERENCES

1. Felix, A., and Pitt, R. M. A New Antigen of *B. typhosus;* Its Relation to Virulence and to Active and Passive Immunisation. *Lancet,* 227:186–191, 1934.

2. Felix, A., Krikorian, K. S., and Reitler, R. The Occurrence of Typhoid Bacilli Containing Vi Antigen in Cases of Typhoid Fever and of Vi Antibody in Their Sera. *J. Hyg.,* 35:421–427, 1935.

3. Horgan, E. S. Notes on the Vi Antigen of *Bacillus typhosus. J. Hyg.,* 36:368–380, 1936.

4. Almon, L., and Stovall, W. D. A Study of the Organisms and Serums from a Number of Typhoid Patients and Carriers. *Am. J. Clin. Path.,* 6:476–486, 1936.

5. Almon, L., Read, J., and Stovall, W. D. A Study of the Vi Antigenic Fraction of Typhoid Bacilli Isolated from Carriers and Cases, and the Antibody Content of the Serum of These Patients. *A.J.P.H.*, 27:357–362, 1937.

6. Bhatnagar, S. S. Vi Agglutination in the Diagnosis of Typhoid Fever and the Typhoid Carrier Condition. *Brit. M. J.*, 2:1195–1198, 1938.

7. Eliot, C. P. The Vi Agglutination Test as an Aid in the Detection of Chronic Typhoid Carriers. *Am. J. Hyg.*, 31:8–15 (Sec. B), 1940.

8. Crossley, V., MacAlpine, J., and Conway, H. Preliminary Report on Vi Agglutination as a Routine Diagnostic Test. *Canad. Pub. Health, J.*, 31:18–19, 1940.

9. Almon, L., and Stovall, W. D. Further Study of the Vi Antibody Content of the Sera of Typhoid Patients and Carriers. *J. Lab. & Clin. Med.*, 25:844–848, 1939–40.

10. Horgan, E. S., and Drysdale, A. Vi Agglutination in Detection of Typhoid Carriers. *Lancet*, 238: 1084–1085, 1940.

11. Pijper, A., and Crocker, C. G. The Agglutinins of Typhoid Carriers. *J. Hyg.*, 37:332–339, 1937.

12. Bhatnagar, S. S., Speechly, C. G. J., and Singh, M. A Vi Variant of *Salmonella typhi* and its Application to the Serology of Typhoid Fever. *J. Hyg.*, 38:663–672, 1938.

13. Feliz, A. Detection of Chronic Typhoid Carriers by Agglutination Tests. *Lancet*, 235:738–741, 1938.

14. Eliot, C. P., and Cameron, W. R. Epidemiological Investigation of Rural Typhoid with the Aid of the Vi Agglutination Test. *A.J.P.H.*, 31:599–604, 1941.

15. Shibley, G. S. Studies in Agglutination. IV. The Agglutination Inhibition Zone. *J. Exper. Med.*, 50:825–841, 1929.

16. Kauffmann, F. Untersuchungen über die Körperantigene in der Salmonella-Gruppe. *Ztschr. f. Hyg. u. Infektionskr.*, 117:778–791, 1935–36.

17. Craigie, J. Personal communication.

18. McFarland, J. The Nephelometer: An Instrument for Estimating the Number of Bacteria in Suspensions Used for Calculating the Opsonic Index and for Vaccines. *J.A.M.A.*, 49:1176–1178, 1907.

Important News

In response to numerous inquiries, the Program Committee announces that plans are proceeding to hold the 71st Annual Meeting of the Association in St. Louis, October 27–30 as planned.

Public health is sufficiently identified with the war effort to make it imperative that the profession should hold its regular session, to which will come many members of the armed forces of the United Nations and many leaders in war industry to share the latest information available both here and abroad for our common purpose. There will be representation from England, where recent experience shows that it is essential that public health groups meet—not in spite of the emergency, but because of it, and because they are so integral a part of defense.

As Dr. Parran has so well said, "Ours is the responsibility for leading the fight against the weakness from within which impedes attack upon the enemy without."